



Research Article

Validation method for determining sodium benzoate in fruit juice drinks in Malang

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ABSTRACT

Sodium benzoate is a preservative that is permitted to be used in food and beverages. The maximum limit for using sodium benzoate in food is 600 mg/L of ingredients calculated as benzoic acid. In this study, High-Performance Liquid Chromatography (HPLC) method using a PDA detector was used for sodium benzoate levels analysis in fruit juice drink circulating in Malang. The mobile phase composition used in this study was methanol pro-HPLC:aqua pro injection (70:30) and added glacial acetic acid to reach pH 3. The analysis was conducted at a wavelength of 245 nm, and the flow rate was set at 1.00 mL/minute. This method was validated against the parameters of selectivity, linearity, accuracy, and precision, and the results obtained to meet the validation requirements. The application of the HPLC method after being validated in the determination of sodium benzoate levels in fruit juice drink circulating in Malang resulted in selectivity $R_s \geq 1.5$, linearity obtained in the range 0.1-5 µg/mL with a correlation coefficient (r) = 0.9999, recovery at a concentration of 80%, 100%, and 120% was 105.31%, and precision with an RSD value of 1.40%. Based on the results, it can be concluded that the validation method of analysis shows that the method can be used to determine sodium benzoate level using HPLC with PDA detectors for routine use in fruit juice drink samples.

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INTRODUCTION

Fruit juice drink, according to SNI 01-3719-1995, is a soft drink made from fruit juice with the addition of sugar or not and with the addition of food additives that are permitted. These additives can be in the form of flavoring, coloring, antioxidants, preservatives, sweeteners, thickener, and regulators to the acid. The use of additional ingredients in

beverages can improve the quality of a product so that it can attract the attention of the community with a delicious and refreshing taste so that they can compete in the market. Additional ingredients in fruit juice drink that often used as preservatives. The purpose of adding preservatives to fruit juice drinks is to prevent or inhibit the decomposition of microorganisms to drinks and to prevent the growth of bacteria and fungi (Isran, 2016).

One of the most used preservatives is benzoic acid. This preservative is used more in its salt form because of its solubility better than the acid form. The most commonly used form of salt from benzoate acid is sodium benzoate which works effectively at pH 2.5-4, so it is widely used in food and beverages and is very suitable for fruit juices and acidic soft drinks (Suryaningrum, 2017; Wati & Guntarti, 2012). The permissible levels of sodium benzoate in soft drinks are 600 mg/kg (Badan Pengawas Obat dan Makanan (BPOM), 2013).

Excessive addition of sodium benzoate can lead to hypertension in consumers with asthma. Besides, the addition of sodium benzoate mixed with oxidizing agents can cause cancer will produce benzene which can cause cancer. These chemicals can cause DNA damage in the mitochondria (Maga & Tu, 1995).

Some analyzes that have been used include the HPLC method on carbonated drinks (Rahmawati, Kosman, Efendi & Ismayani, 2014), UV-Vis Spectrophotometry method in soy milk (Rustian, Rusti & Risnadi, 2015), and the Titration method in analysis sodium benzoate in tomato sauce (Yulinda, 2015).

The analysis of sodium benzoate in fruit juice drinks in this study used the High-Performance Liquid Chromatography (HPLC) method because analysis with HPLC was fast, excellent separation, sensitive, easy sample preparation, and could connect with the appropriate detector (Gandjar & Rohman, 2012). The analytical method that will develop can only use if it has validated, so that accurate results obtained. It is to ensure that when the routine analysis is carried out, the method will be accurate, specific, and have good repetition (Yuwono & Indrayanto, 2005). In this study, the validation method will be carried out on testing additional ingredients contained in fruit juice drinks circulating in Malang, which is sodium benzoate as preservative using HPLC.

RESEARCH METHOD

Material

Sodium benzoate pharmaceutical grade (Brataco Chemical), guava fruit juice drink, aqua pro injection (Ika Pharmindo), acetic acid glacial pro analysis (Merck.), and methanol pro HPLC (Merck.).

Instrument

HPLC (Shimadzu LC-6A), UV Detector, Luna 5u C18(2) 100A column (4,6 X 250), syringe filter membrane, analytical balance (Mettler Toledo AL20), glassware, eluent filter 0.45 μ m, and sample filter 0.2 μ m (Agilent).

Methods

Sodium Benzoate Standard Solution
The sodium benzoate standard solution was prepared to contain 1000 ppm. Working standard solution made with a concentration of 0.1; 0.2; 0.5; 1; and 5 mg/mL from the sodium benzoate standard solution.

Sample Preparation

From 120 mL packaged fruit juice drinks, pipette 5.0 ml and then added distilled water until 10.0 ml, shaken until homogeneous, and then filtered with membrane filter. The sample replicated three times.

HPLC Optimization

The mobile phase composition used is methanol pro HPLC:aqua Pro Injection (70:30) and added acetic acid glacial upto pH 3. The wavelength of the analyst is 245 nm (Altiokka, Ergun, Nafiz & Aboul-Enim, 2015). The flow rate in the HPLC instrument was set to 1.00 mL/minute.

Validation Test

- Selectivity

The selectivity test for sodium benzoate was carried out by comparing the same (identical) retention time (Rt) from the chromatogram at the injection of the sample solution with the comparable standard solution of sodium benzoate in the same HPLC condition. Samples were stated to contain sodium benzoate if peaks appear at the same maximum wavelength, Rt, and spectral pattern as the comparable standard solution of sodium benzoate. Selectivity also expressed by the value of resolution or separation (Rs), and its value 1.5.

- Linearity

The standard working solution of sodium benzoate was injected as much as 20 μ L into the HPLC system. The chromatogram was recorded, a calibration curve was made, and then the regression equation and correlation coefficient were calculated. A calibration curve was created by plotting the peak area on the y-axis and concentration on the x-axis. Then the r value analysis was performed. If the calculated r value is higher than r table (0.05), then the calculated r value meets the requirements.

- Accuracy

Accuracy expressed as the ratio between the results obtained with the actual results, which is



Figure 1. Sodium benzoate spectra on standard (A) and sample (B) solutions.

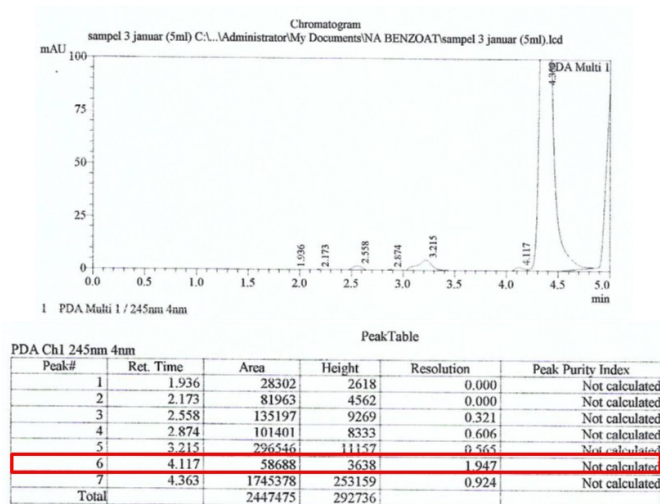


Figure 2. Fruit juice drink sample chromatogram.

Table 1. The result of linearity test.

Concentration	Peak Area
0.1095	16532
0.2190	21067
0.5475	46999
1.0950	93315
5.4750	466050

the concentration of 80%, 100%, and 120%, and each concentration replicated three times.

• Precision

Precision shows the degree of conformity between individual test results carried out by injecting 10 ppm sodium benzoate solution, which has replicated six times (Harmita, 2005).

Determination of sodium benzoate concentration in fruit juice drinks

A solution of 20 μ l fruit juice drink samples injected into the HPLC system with the same conditions as in the standard solution of sodium benzoate. The sample peak area data entered into the standard solution regression line equation ($y = a + bx$) so that the levels of sodium benzoate can obtain in the sample.

RESULTS AND DISCUSSION

Methods validation to determining the level of sodium benzoate by HPLC

Selectivity

Selectivity can be shown from the similarity of the spectra produced between the standard solution of sodium benzoate and the sample solution and produces the same maximum wavelength (Figure 1).

In the chromatogram, it showed that the peak of the standard solution has same Rt (4.082) as the sample (4.117). The selectivity of a method can also show from the resolution value between sodium benzoate and other ingredients in fruit juice drinks that have a good resolution value of ≥ 1.5 (Figure 2) (Harmita, 2005).

Linearity

The results of the linearity test (Table 1) obtained from the calibration curve obtained with five variations of concentration (0.1; 0.2; 0.5; 1; and 5 μ g/ml). The results of the linear regression equation obtained are $(r) = 0.9999$, with the equation $Y = 84465.86 x + 3006.04$. The results of linearity calculations show that the value of r analyte > 0.999 . It can be concluded that the analyte provides a linear response between the concentration with the peak area.

Accuracy

An accuracy test was carried out by analyzing placebo samples added (addition) of Sodium Benzoate with three different concentration levels. Each concentration replicated three times. Accuracy

Table 2. The result of accuracy test.

Concentration	Replication	Amount Added (g)	Amount Obtained (μg)	Recovery (%)
80 %	1	0.00809	0.00904	111.74
	2	0.00809	0.00913	112.85
	3	0.00809	0.00921	113.84
100 %	1	0.01008	0.01012	100.40
	2	0.01008	0.01018	100.99
	3	0.01008	0.01018	100.99
120 %	1	0.01175	0.01218	103.66
	2	0.01175	0.01191	101.36
	3	0.01175	0.01198	101.96
% Recovery Average				105.31 \pm 5.72

Table 3. The result of precision test.

Replication	Rt	Area
1	4.080	1026475
2	4.074	1002753
3	4.085	1003905
4	4.076	1026182
5	4.075	1038321
6	4.092	1027188
Average		1020804 \pm 14283.5
% RSD		1.40

Table 4. The result of determination of sodium benzoate in fruit juice drink.

Replication	Area	Concentration
1	65559	1.4812 $\mu\text{g/ml}$
2	64972	1.4672 $\mu\text{g/ml}$
3	58688	1.3184 $\mu\text{g/ml}$
Average		1.4223 \pm 0.09

test results stated in % recovery (Table 2). The accuracy test results obtained showed that the average % of sodium benzoate recovery = 105.31 \pm 5.72. The concentration of multilevel standard solutions added recommended as 80-120% or 75-125% of the target analyte concentration (International Conference of Harmonization, 1996; Yuwono and Indrayanto, 2005). So the accuracy of sodium benzoate meets the accuracy requirements.

Precision

The precision test was carried out by subjecting sodium benzoate in a placebo matrix solution, which was standardized and replicated six times (Table 3)

The precision test results showed that the RSD value for sodium benzoate was 1.40%. Precision requirements are RSD \leq 2% (Harmita, 2005). So that it can conclude that the analysis method meets the precision criteria.

Concentration determination

Determination of the concentration of sodium benzoate in fruit drink juice samples was carried out three times replication on one brand circulating in the city of Malang. The calculation was done by entering the area value into the standard curve regression equation obtained from the linearity test (Table 4).

From the results of the assay where the concentration found in the sample as much as 1.4223 $\mu\text{g/ml}$ is equivalent to 1.4223 mg/L. While the terms of use of sodium benzoate according to BPOM (2013), which is a maximum of 600mg/kg, the preservatives used in fruit juice drinks are still far below the stipulated requirements, so they are safe for consumption by the public.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the validation of the method of analysis with selectivity, linearity, accuracy, and precision shows that the method has been compliant with requirements so that it can be used to determine sodium benzoate using HPLC with PDA detectors for routine use in fruit drink juice samples.

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